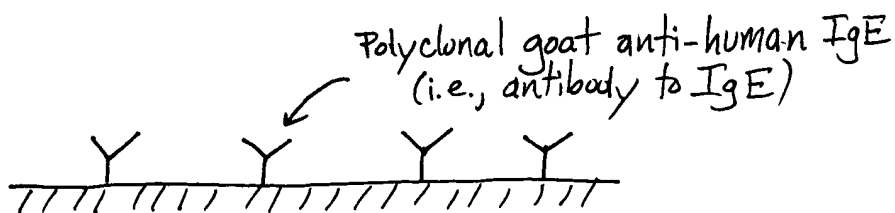


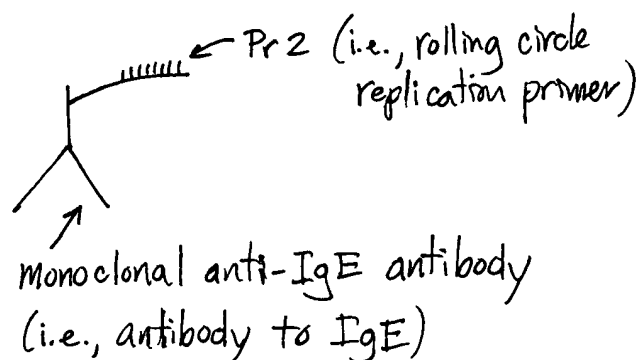
# Appendix A Kingsmore et al. Example 7

## Materials:

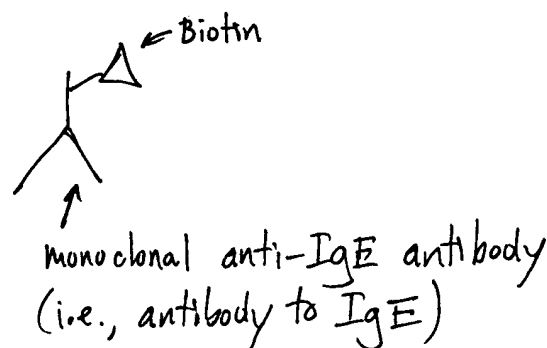
- (a) Microarray:  
(lines 4-8)



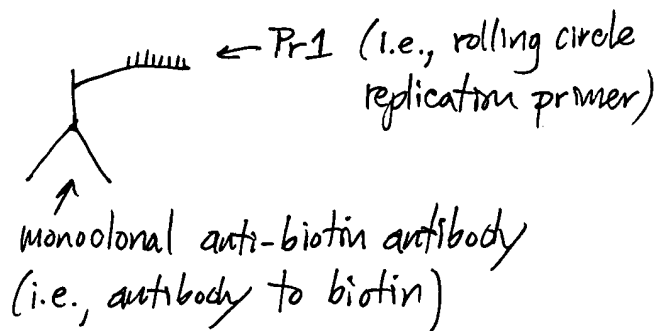
- (b) First conjugate:  
(Pr2 ~ Mab Anti-IgE)  
(lines 8-12)



- (c) Second conjugate:  
(biotin ~ Mab Anti-IgE)  
(lines 8-9 & 12-13)



- (d) Mab Anti-biotin conjugate:  
(lines 14-18)



- (e) Circle 1 (for Pr1): (1)  
(lines 24-25)

- (f) Circle 2 (for Pr2): (2)  
(lines 25)

## Method

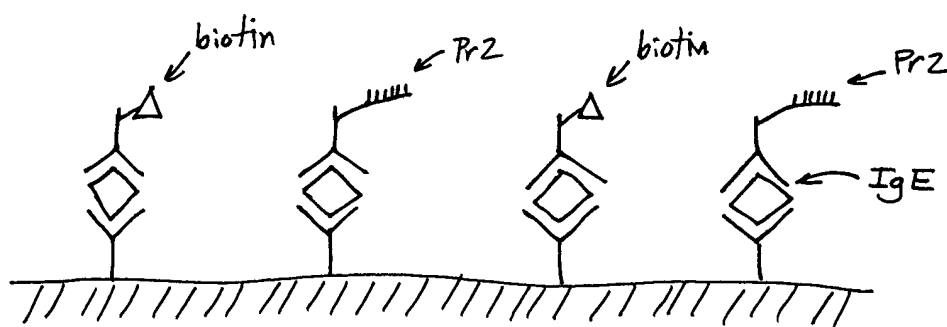


- ① Antigen-antibody complexes pre-formed in separate reactions  
(lines 18-21)

biotin ~ Mab Anti-IgE  $\oplus$  IgE in one tube

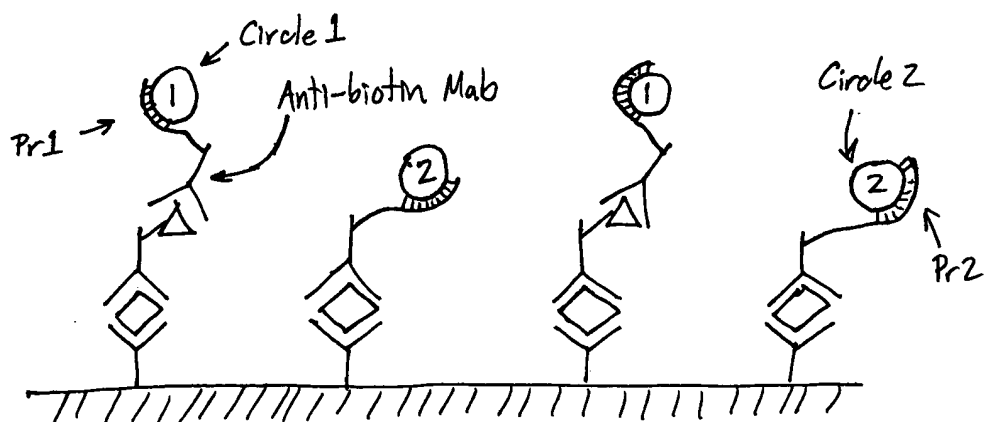
Pr2 ~ Mab Anti-IgE  $\oplus$  IgE in a second tube

- ② Mix antigen-antibody complexes and apply mixtures to arrays (lines 21-24)



- ③ Apply mixture of Circle 1, Circle 2 and Pr1 ~ Mab Anti-biotin to microarrays (lines 24-27)

Note: Circles now associated with antibody-primer conjugates



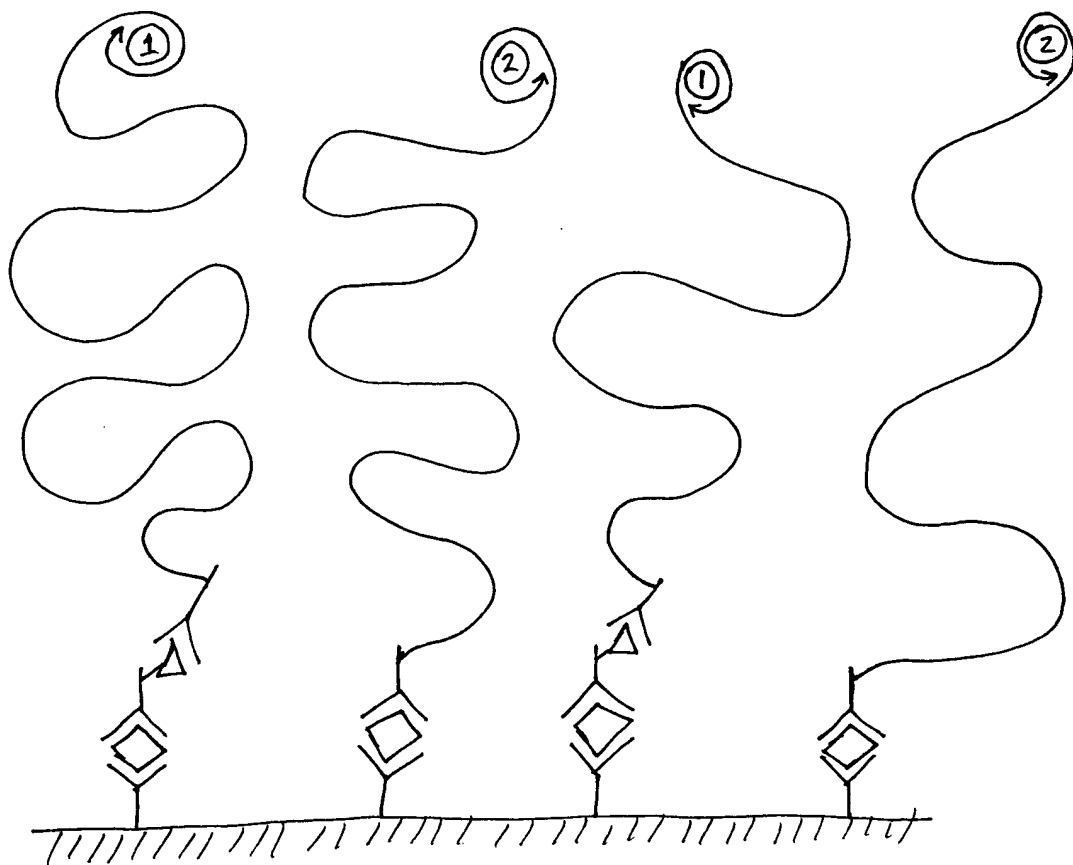


- ④ Wash slides [to remove unbound components]  
(lines 27-28)

Note: Circles associated with antibody-primer conjugates are not dissociated in this step (otherwise, rolling circle amplification would not take place)

- ⑤ Apply RCA reaction mix (buffer, labeled detector probes, polymerase, dNTPs) and incubate [rolling circle amplification of Circle 1 and Circle 2 primed from Pr1 and Pr2, respectively]  
(lines 28-33)

Note: Circles are still associated with antibody-primer conjugates



- ⑥ Wash, dry and scan slides [to detect labeled probes]